JOURNAL OF BACTERIOLOGY, Apr. 2005, p. 2513–2518 Vol. 187, No. 7
0021-9193/05/$08.00+0 doi:10.1128/JB.187.7.2513–2518.2005

Interaction between Individual Protein Components of the GerA and GerB Nutrient Receptors That Trigger Germination of *Bacillus subtilis* Spores

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Received 20 October 2004/Accepted 29 December 2004

Germination of *Bacillus subtilis* spores via the GerA nutrient receptor was suppressed by GerAC lacking the diacylglycerylated cysteine essential for receptor function. Overexpression of the C protein of the GerB nutrient receptor also suppressed the function of both the GerA receptor and a variant GerB receptor, GerB*. These findings suggest that GerAC and GerBC interact with their respective A and B proteins in GerA or GerB receptors and that GerBC potentially interacts with GerAA-GerAB. However, GerAC did not appear to interact with GerBA-GerBB.

Spores of *Bacillus* species are metabolically dormant and can remain so for long periods of time. However, nutrient receptors located in the dormant spore's inner membrane can sense the presence of exogenous nutrients and trigger the process of spore germination that leads to a resumption of metabolism in spore outgrowth (7, 13, 14, 15, 20, 21, 26). There are three functional nutrient receptors in *Bacillus subtilis* spores, the products of the homologous tricistronic *gerA*, *gerB*, and *gerK* operons (termed *gerA* operon homologs) (13, 14, 15, 21, 25, 32). Based on their amino acid sequences, the A and B proteins encoded by *gerA* operon homologs are most likely integral membrane proteins (13, 21, 32). The third encoded protein, C, is probably not an integral membrane protein, but the C proteins have a signal peptide followed by a consensus sequence for diacylglycerol addition to a cysteine residue and a signal peptidase II cleavage site (8, 13, 21, 23, 32). Consequently, it is likely that some combination of the signal peptide and diacylglycerol addition ensures that the C proteins are also in the spore's inner membrane.

The addition of diacylglycerol to the C proteins of GerA receptor homologs is important for receptor function, as loss of the only *B. subtilis* prelipoprotein diacylglycerol transferase, Lgt (also called GerF), reduces significantly the spore germination with nutrients (8, 10, 23). The lack of diacylglycerol addition to C proteins almost completely eliminates the function of the GerA receptor that responds to L-alanine, significantly but not completely abolishes spore germination via the GerB receptor, but has little effect on germination via the GerK receptor (8).

The presence of genes for the A, B, and C proteins of GerA receptor homologs in an operon suggests that each receptor is a complex of the appropriate A, B, and C proteins, and loss of any cistron of a particular *gerA* operon homolog eliminates the function of the encoded receptor (13, 14, 15). While there is as yet no biochemical evidence for physical interaction between the A, B, and C proteins of any individual receptor, there is genetic evidence that GerAA interacts with GerAB and that GerBA interacts with GerBB (15, 18). However, there is no evidence for the physical interaction of the C protein with the A and B proteins of either of these receptors. It was shown recently that substitution of alanine for the diacylglycerylated cysteine residue in GerAC (residue 18) or GerBC (residue 20) reduces GerA and GerB receptor function 200- and 7-fold, respectively (8). One possible explanation for this result is that without diacylglycerol addition GerAC and GerBC do not localize to the spore's inner membrane, leading to lack of assembly or stability of the appropriate A and B proteins. While this explanation has not been dismissed for the GerA receptor, it is not true for the GerB receptor, as GerBA is at normal levels in the inner membrane of *lgt* spores (8). In addition, the GerK receptor functions normally in *lgt* spores, as does GerKC in which alanine has replaced the diacylglycerylated cysteine, although the loss of *gerKC* eliminates GerK receptor function (8). These findings suggest that the GerB and GerK receptors, and by inference the GerA receptor, are in the inner spore membrane even if the receptor's C proteins are not diacylglycerylated. Indeed, a number of membrane proteins that are normally diacylglycerylated are in the plasma membrane of growing *lgt* cells, although the functions of these proteins are often compromised (9, 10, 30). An alternative explanation for the reduced function of the GerA and GerB receptors that are not diacylglycerylated, therefore, is simply that this modification is essential for normal GerA and GerB receptor function. If this alternative explanation is correct, then *gerAC* or *gerBC* in which an alanine codon has replaced the diacylglycerylated cysteine codon might be dominant negative to the wild-type allele if all three proteins encoded by the *gerA* or *gerB* operons physically interact. This communication presents results in support of this prediction.

Plasmid and strain construction. All work was carried out with *B. subtilis* strains (Table 1) derived from and isogenic with strain PS832, a prototrophic derivative of strain 168. Only outlines of plasmid construction in *Escherichia coli* and gener-

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TABLE 1. *B. subtilis* strains used in this study

Strain	Genotype and phenotype[a]	Source or reference
FB10	*gerBB1**	18
FB72	Δ*gerA* Δ*gerB* Δ*gerK* Cm^r Sp^r Tc^r	19
PS832	Wild type	Laboratory stock
PS3604	$gerBC^{Cys20}$ Sp^r	8
PS3605	$gerBC^{Ala20}$ Sp^r	8
PS3608	Δ*gerA* Cm^r	8
PS3611	Δ*gerB* Δ*gerK* Cm^r Em^r Sp^r	8
PS3612	$gerAC^{Ala18}$ Δ*gerB* Δ*gerK* Cm^r Em^r Sp^r	8
PS3629	Δ*amyE*::(P_{gerA}-$gerAC^{Cys18}$) $gerAC^{Ala18}$ Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3630	Δ*amyE*::(P_{gerA}-$gerAC^{Ala18}$) Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3631	Δ*amyE*::(P_{sspB}-$gerAC^{Cys18}$) $gerAC^{Ala18}$ Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3632	Δ*amyE*::(P_{sspB}-$gerAC^{Ala18}$) Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3633	Δ*amyE*::(P_{sspB}-$gerAC^{Cys18}$) Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3636	Δ*amyE*::(P_{sspB}-$gerAC^{Ala18}$) *gerBB1** Km^r	This work
PS3637	Δ*amyE*::(P_{sspB}-$gerAC^{Cys18}$) *gerBB1** Km^r	This work
PS3656	*gerBB1** $gerBC^{Cys20}$ Sp^r	This work
PS3657	*gerBB1** $gerBC^{Ala20}$ Sp^r	This work
PS3658	Δ*amyE*::(P_{sspB}-$gerBC^{Cys20}$) *gerBB1** Km^r	This work
PS3659	Δ*amyE*::(P_{sspB}-$gerBC^{Ala20}$) *gerBB1** Km^r	This work
PS3660	Δ*amyE*::(P_{sspB}-$gerBC^{Cys20}$) Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3661	Δ*amyE*::(P_{sspB}-$gerBC^{Ala20}$) Δ*gerB* Δ*gerK* Cm^r Em^r Km^r Sp^r	This work
PS3662	Δ*amyE*::(P_{sspB}-$gerBC^{Cys20}$) Δ*gerA* Cm^r Km^r	This work
PS3663	Δ*amyE*::(P_{sspB}-$gerBC^{Ala20}$) Δ*gerA* Cm^r Km^r	This work
PS3692	Δ*amyE*::(P_{gerA}-$gerBC^{Cys20}$) Δ*gerA* Cm^r Km^r	This work
PS3693	Δ*amyE*::(P_{gerA}-$gerBC^{Ala20}$) Δ*gerA* Cm^r Km^r	This work
PS3694	Δ*amyE*::(P_{gerA}-$gerBC^{Cys20}$) *gerBB1** Km^r	This work
PS3695	Δ*amyE*::(P_{gerA}-$gerBC^{Ala20}$) *gerBB1** Km^r	This work

[a] Cm^r, resistance to chloramphenicol (5 μg/ml); Em^r, resistance to erythromycin (1 μg/ml) and lincomycin (25 μg/ml); Km^r, resistance to neomycin (7 μg/ml); Sp^r, resistance to spectinomycin (100 μg/ml); Tc^r, resistance to tetracycline (10 μg/ml).

ation of *B. subtilis* strains by transformation with linearized plasmid DNA are given; details are available on request. PCR and DNA sequence analyses confirmed the chromosomal structures of *B. subtilis* transformants.

Strains expressing $gerAC^{Cys18}$ (wild-type allele with Cys at position 18) or $gerAC^{Ala18}$ at the *amyE* locus from the *gerA* promoter (P_{gerA}) were generated as follows. A Km^r cassette from plasmid pDG780 (6) was inserted into plasmid pTEF1/Zeo (Invitrogen, Carlsbad, Calif.), giving plasmid pTI1. A ~400-bp fragment including P_{gerA} and the ribosome-binding and translation start sites of the *gerAA* cistron was PCR amplified from chromosomal DNA of strain PS3611. The sequence around the *gerAA* translation start site in this fragment was 5′-GGTGAC CCAT**ATG**AGA-3′ (the initiation codon is shown in boldface type) instead of 5′-GGTGACCTCA**TTG**GAA-3′ as in wild-type *gerAA*. The spacing between the ribosome-binding site and the translation initiation codon is the same in both cases, but the translation initiation codon and the three nucleotides upstream of the initiation codon differ. This fragment was inserted in plasmid pTI1, giving plasmid pTI2. A 1.3-kb fragment including the *gerAC* translation start site and coding sequence and extending beyond the likely *gerA* operon's transcription terminator was amplified from strains PS3611 and PS3612 (5, 32). These fragments were inserted in plasmid pTI2, giving plasmids pTI3a (fragment from PS3611) and pT13b (fragment from PS3612). Fragments (3.2 kb) containing P_{gerA} upstream of *gerAC* from pTI3a and pTI3b were inserted in plasmid pDG364 (4), giving plasmids pTI4a (from pTI3a) and pTI4b (from pTI3b). The transformation of *B. subtilis* strain PS3612 with pTI4a gave strain PS3629, and the transformation of strain PS3611 with pTI4b gave strain PS3630.

The construction of strains overexpressing *gerAC* at *amyE* from the strong forespore-specific *sspB* promoter (P_{sspB}) (12, 29) was as follows. A ~400-bp fragment including P_{sspB} and up to the *sspB* translation initiation codon was amplified from chromosomal DNA of strain PS832. This fragment was combined with most of plasmids pTI6a and pTI6b (derived from pTI4a and pTI4b, respectively), giving plasmids pTI7a (from pTI6a) and pTI7b (from pTI6b). The transformation of strains PS3611 and PS3612 with pTI7a gave strains PS3633 and PS3631, respectively; the transformation of strain PS3611 with pTI7b gave strain PS3632. Previous work (1, 12, 29) suggests that the level of GerAC overexpressed from P_{sspB} will be ~100-fold higher than when *gerA* is expressed from P_{gerA}.

To test the effects of GerAC overexpression on spore germination via the GerB receptor, we used spores of strains carrying a missense mutation in *gerBB* termed *gerBB1**; the encoded GerB* receptor is sufficient to trigger spore germination in response to D-alanine, and even better with D-alanine plus D-glucose or with L-asparagine (1, 18). The transformation of strain FB10 with pTI7a and pTI7b gave strains PS3636 and PS3637, respectively. The transformation of strain FB10 with plasmids pBC^{C20} and pBC^{A20} (8) gave strains PS3656 and PS3657, respectively.

B. subtilis strains overexpressing $gerBC^{Cys20}$ or $gerBC^{Ala20}$ at *amyE* from P_{sspB} were constructed as follows. The *gerBC* gene was amplified from chromosomal DNA of strains PS3604 and PS3605; the fragments were combined with most of pTI7a, giving plasmids pTI8a ($gerBC^{Cys20}$) and pTI8b ($gerBC^{Ala20}$) in which *gerBC* transcription is from P_{sspB}. Transformation of strains FB10 and PS3611 by pTI8a gave strains PS3658 and PS3660, respectively; transformation of strains FB10 and

TABLE 2. Germination of spores of various strains via the GerA receptor[a]

Strain	Genotype	CFU/12 h	Rate of DPA release (% of maximum value)
FB72	Δ*gerA* Δ*gerB* Δ*gerK*	1.5×10^5	<2
PS832		1.5×10^8	104
PS3611	Δ*gerB* Δ*gerK*	4.1×10^7	100[b]
PS3612	$gerAC^{Ala18}$ Δ*gerB* Δ*gerK*	1.5×10^5	<2
PS3629	Δ*amyE*::(P_{gerA}-$gerAC^{Cys18}$) $gerAC^{Ala18}$ Δ*gerB* Δ*gerK*	1.4×10^7	25
PS3630	Δ*amyE*::(P_{gerA}-$gerAC^{Ala18}$) Δ*gerB* Δ*gerK*	4.7×10^7	104
PS3631	Δ*amyE*::(P_{sspB}-$gerAC^{Cys18}$) $gerAC^{Ala18}$ Δ*gerB* Δ*gerK*	1.5×10^8	360
PS3632	Δ*amyE*::(P_{sspB}-$gerAC^{Ala18}$) Δ*gerB* Δ*gerK*	7×10^6	10
PS3633	Δ*amyE*::(P_{sspB}-$gerAC^{Cys18}$) Δ*gerB* Δ*gerK*		470
PS3660	Δ*amyE*::(P_{sspB}-$gerBC^{Cys20}$) Δ*gerB* Δ*gerK*	3.4×10^5	9
PS3661	Δ*amyE*::(P_{sspB}-$gerBC^{Ala20}$) Δ*gerB* Δ*gerK*	4.2×10^7	115

[a] Spores were prepared, cleaned, and tested for colony-forming ability and for DPA release upon germination with L-alanine as described in the text. CFU values are the averages of duplicate determinations with two independent spore preparations; individual values differed by ≤25% from the average value. DPA release rates are the averages of duplicate measurements with two independent spore preparations; individual values differed by ≤25% from the average values.

[b] This value was set at 100%.

PS3611 by pTI8b gave strains PS3659 and PS3661, respectively (Table 1). The transformation of strain PS3608 with chromosomal DNA of strains PS3660 and PS3661 gave strains PS3662 and PS3663, respectively. Since GerBA levels in spores with the *gerB* or *gerB** operons transcribed from P_{sspB} were 200- to 500-fold higher than wild-type GerB levels (1, 20), levels of GerBC overexpressed from P_{sspB} will also likely be elevated 200- to 500-fold (2, 5, 12, 32).

For construction of *B. subtilis* strains overexpressing $gerBC^{Cys20}$ or $gerBC^{Ala20}$ at *amyE* from P_{gerA}, P_{gerA} from plasmid pTI6a replaced P_{sspB} in pTI8a and pTI8b, giving plasmids pTI9a (from pTI8a) and pTI9b (from pTI8b). The transformation of strains PS3608 and FB10 with these plasmids gave strains PS3692 and PS3694 (from pTI9a), respectively, and PS3693 and PS3695 (from pTI9b), respectively.

Spore preparation and measurement of spore germination. Spores of various strains were prepared on 2× Schaeffer's glucose medium agar plates without antibiotics at 37°C and cleaned and stored as described previously (16, 17). All spore preparations used were free (>98%) from growing or sporulating cells or cell debris, as determined by phase contrast microscopy.

Spore germination was measured in two ways. In one, spores at an optical density at 600 nm (OD_{600}) of 20 were heat shocked (30 min; 70°C) and cooled on ice. The heated spores were diluted in water, aliquots were spotted on Luria-Bertani medium agar plates (19) without antibiotics, and colonies appearing after 12 h at 37°C were counted. Since the *gerA* operon homologs are expressed only during sporulation (13, 21), mutations in these operons have no effects on cell growth or spore outgrowth, and this assay measures only differences in rates of spore germination (8, 19).

In the second assay, heat-shocked spores at an OD_{600} of 1.5 were germinated at 37°C in (i) 1 mg of L-alanine/ml–6.95 mg (each) of KH_2PO_4 and $NaHPO_4$ (pH 7.25)/ml–100 mM NaCl–200 mM KCl, (ii) 10 mM D-alanine–25 mM Tris-HCl (pH 8), (iii) 10 mM D-alanine–10 mM D-glucose–25 mM Tris-HCl (pH 8), (iv) 3 mM L-asparagine–25 mM Tris-HCl (pH 8), or (v) 3 mM L-asparagine–500 μg of D-glucose/ml–500 μg of D-fructose/ml–50 mM KPO_4 (pH 7.4) (AGFK) (1, 8, 18). At various times, aliquots (1 ml) of the suspensions were centrifuged for 2 min in a microcentrifuge and the OD_{270} of the supernatant fluid was determined. This assay measures the release of the spore core's depot of pyridine-2,6-dicarboxylic acid (dipicolinic acid [DPA]), an early event in spore germination (1, 24, 26). Ninety percent or more of the OD_{270} released by germinating *B. subtilis* spores is due to DPA, as the UV spectrum of the material released is identical to that of pure DPA (data not shown), as expected based on previous work (22). Maximum rates of DPA release were calculated as described previously (1, 24).

Effects of $GerAC^{Ala18}$ on spore germination via the GerA receptor. Analysis of the germination of spores of various strains by determination of colonies formed from spores applied to Luria-Bertani medium plates (Table 2) showed that germination of spores containing only the GerA receptor (strain PS3611) was ~4-fold lower than that of wild-type spores (strain PS832). Germination of spores lacking all nutrient receptors (strain FB72) or lacking the GerB and GerK receptors and with a GerA receptor containing $GerAC^{Ala18}$ (strain PS3612) was greatly decreased in this assay (Table 2), as found previously (8, 18). When spores lacked the GerB and GerK receptors and contained both $gerAC^{Cys18}$ and $gerAC^{Ala18}$, at either *amyE* or *gerA* (strains PS3629 and PS3630), there was significant spore germination. However, spores of strains with the *gerAC* at *amyE* expressed from P_{gerA} exhibited different rates of germination depending on the locations of the wild-type and mutant *gerAC* cistrons. Spores of the strain with $gerAC^{Cys18}$ in the *gerA* operon and $gerAC^{Ala18}$ expressed from P_{gerA} at *amyE* (strain PS3630) germinated more rapidly than spores of the strain with the positions of the *gerAC* alleles reversed (strain PS3629). The same general result was obtained when *gerAC* alleles at *amyE* were overexpressed from P_{sspB} (~100-fold stronger than P_{gerA}) (29) (strains PS3631 and PS3632). However, while overexpression of $gerAC^{Ala18}$ reduced spore germination significantly (strain PS3632), overexpression of $gerAC^{Cys18}$ increased spore germination (strains PS3631 and PS3633) (Table 2).

The results from assays of spore germination by colony formation were confirmed when rates of spore germination with

TABLE 3. Germination of spores of various strains via the GerB or GerB* receptor[a]

Strain	Genotype	Rate of spore germination (% of maximum) with:[b]			
		AGFK	D-ala	D-ala/D-glu	L-asn
FB10	*gerBB1**		100[c]	100[c]	100[c]
PS3608	*gerA*	100[c]			
PS3636	*amyE*::(P_{sspB}-*gerAC*^{Ala18}) *gerBB1**		71	64	68
PS3637	*amyE*::(P_{sspB}-*gerAC*^{Cys18}) *gerBB1**		80	91	85
PS3656	*gerBB1** *gerBC*^{Cys20}		104	103	97
PS3657	*gerBB1** *gerBC*^{Ala20}	2	9	8	9
PS3658	*amyE*::(P_{sspB}-*gerBC*^{Cys20}) *gerBB1**		32	16	27
PS3659	*amyE*::(P_{sspB}-*gerBC*^{Ala20}) *gerBB1**		78	55	78
PS3662	*amyE*::(P_{sspB}-*gerBC*^{Cys20}) *gerA*	56			
PS3663	*amyE*::(P_{sspB}-*gerBC*^{Ala20}) *gerA*	63			
PS3692	*amyE*::(P_{gerA}-*gerBC*^{Cys20}) *gerA*	106			
PS3693	*amyE*::(P_{gerA}-*gerBC*^{Ala20}) *gerA*	121			
PS3694	*amyE*::(P_{gerA}-*gerBC*^{Cys20}) *gerBB1**		87	100	121
PS3695	*amyE*::(P_{gerA}-*gerBC*^{Ala20}) *gerBB1**		88	85	112

[a] Spores of different strains were germinated with various agents, germination was measured by the release of DPA, and the maximum rates of germination were calculated as described in the text.

[b] Values are the averages of duplicate measurements on three different spore preparations. Individual values differed by ≤35% from the average values. D-ala, D-alanine; D-glu, D-glucose; and L-asn, L-asparagine.

[c] This value was set at 100%.

L-alanine were measured by DPA release (Table 2). In particular, the overexpression of *gerAC*^{Ala18} at *amyE* (strain PS3632) reduced the rate of spore germination with L-alanine 10-fold, while the overexpression of *gerAC*^{Cys18} at *amyE* (strains PS3631 and PS3633) stimulated the rate of spore germination ~4-fold (Table 2).

Effects of GerBC^{Ala20} on spore germination via the GerB receptor. Previous work has shown that GerB receptor function is significantly decreased when GerBC cannot be diacylglycerylated, as spore germination with AGFK which triggers germination through cooperative action of the GerB and GerK receptors, is reduced ~7-fold in spores carrying *gerBC*^{Ala20}, although the analogous modification of *gerKC* has very little effect (8). Diacylglycerylation of GerBC was also required for GerB* receptor function, as germination of spores of strain PS3657 was reduced ~11-fold compared to that of FB10 or PS3656 spores (Table 3). The overexpression of GerBC^{Ala20} from P_{sspB} (strains PS3663 and PS3659) reduced the germination of *gerA* spores with AGFK and germination of *gerBB1** spores with various germinants, but these reductions were small and not especially significant (Table 3). The overexpression of GerBC^{Cys20} also reduced spore germination slightly via the GerB receptor (strain PS3662); via the GerB* receptor (strain PS3658) it gave even more of a reduction than that given by the overexpression of GerBC^{Ala20} (strains PS3659 and PS3663) (Table 3). In contrast, the expression of either GerBC^{Cys20} or GerBC^{Ala20} from P_{gerA} did not inhibit notably the germination via the GerB or GerB* receptor (strains PS3692, PS3693, PS3694, and PS3695) (Table 3).

Effects of GerAC^{Ala18} or GerBC^{Ala20} on spore germination via the GerB or GerA receptor, respectively. The inhibition of spore germination via the GerA receptor by the overexpression of GerAC^{Ala18} and of germination via the GerB or GerB* receptor by the overexpression of GerBC^{Cys20} or GerBC^{Ala20} suggested that the overexpressed C proteins may sequester some spore components that are limiting for germination in an inactive complex. Such limiting components might be the A and B proteins of the GerA or GerB receptors or other proteins altogether. To distinguish between these possibilities, we examined the effects of overexpressed GerAC on spore germination via the GerB* receptor and the effect of overexpressed GerBC on spore germination via the GerA receptor. The effects of overexpression of GerAC^{Ala18} from P_{sspB} on spore germination via the GerB* receptor (strains PS3636 and PS3637) were small and likely not significant (Table 3), even though the GerB* receptor proteins are likely present in spores at ~10-fold lower levels than the GerA receptor proteins (1, 2, 5). The overexpression of GerAC^{Cys18} (strain PS3637) gave a ≤20% decrease in spore germination with the various nutrients to which the GerB* receptor responds, and there was only a slightly larger decrease upon the overexpression of GerAC^{Ala18} (strain PS3636); while neither of these decreases is likely to be significant, the overexpression of GerBC^{Cys20} decreased germination via the GerA receptor ~11-fold (strain PS3660), although the overexpression of GerBC^{Ala20} had essentially no effect on GerA receptor function (strain PS3661) (Table 2).

Alternative explanations for the results. We had hoped to interpret the results of the experiments described above as due only to alterations in the levels of the various C proteins. However, there are a number of alternative explanations that should be considered, including the following. (i) Previous work has shown that the overexpression of the *gerA* operon abolishes sporulation (1), and even a small alteration in sporulation can alter the germination properties of the resultant spores (25). However, we saw no alteration in sporulation upon overexpression of the various C proteins, except for GerAC^{Ala18}, whose overexpression under the control of P_{sspB} (but not P_{gerA}) reduced sporulation efficiency ~3-fold (data not shown). The reason for the reduced sporulation efficiency is not clear, but the effect was potentially worrisome. However, it seems unlikely that this is the reason for the effects of overexpression of GerAC^{Ala18} on spore germination via the GerA receptor, since (a) GerAC^{Ala18} expressed from P_{gerA} had similar, albeit smaller effects, and (b) GerAC^{Ala18} overexpressed from P_{sspB} had only a slight effect on spore germina-

tion via the GerB* receptor. (ii) Perhaps the C proteins regulate the expression of their own operon. If so, an alteration in C protein levels might alter the level of the complete nutrient receptor. However, there is no evidence for such autoregulation, and the different effects of the C proteins with and without diacylglycerylation upon GerA and GerB receptor function are difficult to fit into such a model. (iii) Perhaps some of the effects of overexpressed C proteins are due to competition for a limiting amount of Lgt, thus reducing diacylglycerylation of other C proteins and altering nutrient receptor function. However, previous work has shown that the overexpression of *gerB* from P_{sspB} has no effect on GerA receptor function (8), suggesting that there is sufficient Lgt to diacylglycerylate overexpressed C proteins. In addition, the overexpression of $GerAC^{Cys18}$ increased germination via the GerA receptor, while this germination pathway was inhibited by the overexpression of $GerAC^{Ala18}$. (iv) Some of the C proteins may be unstable when expressed alone and thus do not accumulate to high levels. This is a reasonable possibility, and as a consequence, the interpretation of experiments in which an overexpressed C protein has no effect may be ambiguous. However, this possibility should not affect the interpretation of experiments in which significant alterations in spore germination accompanied the overexpression of a C protein. (v) The effects of C protein overexpression on spore germination may be masked because the nutrient receptors are not rate limiting for spore germination. We used DPA release as a measure of spore germination, since this is an early event in germination that is independent of later events, such as hydrolysis of the spore cortex (21, 26). In addition, DPA release is the earliest spore germination-associated process that is triggered by nutrients binding to their receptors and is easily measured (26). However, until the precise function of the nutrient receptors is understood and the proteins these receptors interact with are known, this will remain an area of uncertainty.

Conclusions. With the provisos noted above in mind, the results in this communication lead to a number of conclusions. First, since the expression of $gerAC^{Ala18}$ from P_{gerA} had a much greater effect when $gerAC^{Ala18}$ was in the *gerA* operon than when it was expressed monocistronically at *amyE*, GerAA, GerAB, and GerAC may assemble in a complex as these proteins are translated from a polycistronic mRNA. Presumably, GerAC made from mRNA transcribed at *amyE* is less able to compete for binding to GerAA-GerAB than is GerAC translated from polycistronic *gerA* mRNA. However, since wild-type GerAC translated from a monocistronic mRNA transcribed from P_{gerA} at *amyE* was able to suppress the effect of a $gerAC^{Ala18}$ mutation in the intact *gerA* operon, there must be some mixing of GerAC proteins made from various locations, although GerAC lacking diacylglycerol appears to be less able to compete for GerAA-GerAB than the diacylglycerylated protein. However, we assume that P_{gerA} is transcribed equally well at the *gerA* and *amyE* loci, and this may not be the case. Transcription of *gerA* is by RNA polymerase with the sporulation-specific σ factor σ^G, and under some conditions P_{gerA} can be utilized in vivo by $E\sigma^F$ (5, 29). In addition, the *amyE* locus enters the developing spore well before the *gerA* locus (30). Consequently, P_{gerA} at *amyE* may be more frequently transcribed, perhaps even to some degree by $E\sigma^F$, than is P_{gerA} at *gerA* (29, 31). We also cannot be sure that *gerAC* mRNA made as a monocistronic transcript at *amyE* and as a part of the polycistronic transcript at *gerA* are translated equally well, since translational coupling may affect the translation of *gerAC* in the polycistronic mRNA and there are differences between the ribosome-binding regions and translation start codons of *gerAC* mRNA at *amyE* and *gerA*.

The second conclusion follows from the observation that the overexpression of $GerAC^{Cys18}$ significantly increased the rate of spore germination via the GerA receptor. This increase was also seen when the *gerA* operon was overexpressed (1), but it was surprising to obtain this result when only GerAC was overexpressed, since GerAA and GerAB are essential for L-alanine triggering of spore germination via the GerA receptor (13, 14, 15, 21). One possible conclusion is that the levels of these three proteins (normally quite low) (5, 7, 20) and their affinities for one another are such that there is significant dissociation of GerAC from GerAA-GerAB in wild-type spores. Consequently, the overexpression of GerAC may increase GerAA-GerAB-GerAC complex formation, thus increasing GerA-mediated germination. This conclusion is obviously consistent with GerAC physically interacting with GerAA and GerAB (see also below). Since the overexpression of $GerAC^{Ala18}$ or $GerAC^{Cys18}$ altered GerA receptor function, overexpressed GerAC might have interfered with GerB or GerK receptor function, but this was not the case.

The observations noted above for spores with overexpressed $GerAC^{Ala18}$ held true to some degree for overexpressed $GerBC^{Ala20}$, as germination via the GerB or GerB* receptor was decreased by high levels of $GerBC^{Ala20}$. However, the effects of overexpressed $GerBC^{Ala20}$ were small, suggesting that this protein competes poorly for GerBA-GerBB. High levels of $GerBC^{Cys20}$ also decreased germination, in particular via the GerB* receptor, in contrast to the increase in GerA receptor-mediated germination by high levels of $GerAC^{Cys18}$. This finding suggests that the interaction of GerBC with GerBA-GerBB is much stronger than is the comparable interaction in the GerA receptor. However, we have no good explanation for the inhibition of GerB* receptor function by the overexpression of $GerBC^{Cys20}$. Since the level of *gerB* expression is significantly lower than that of *gerA* (2, 5), the ratio of overexpressed GerBC to GerBA-GerBB will likely be much higher than the ratio of overexpressed GerAC to GerAA-GerAB; perhaps the extremely high relative level of $GerBC^{Cys20}$ allows formation of higher oligomers of GerBC with GerBA-GerBB that are inactive. Indeed, the expression of $GerBC^{Cys20}$ from the much weaker P_{gerA} did not reduce significantly the germination via the GerB or GerB* receptors (Table 3). In any case, the finding that the overexpression of $GerBC^{Cys20}$ in particular decreased spore germination due to the GerB* receptor is consistent with the physical interaction of GerBC with GerBA-GerBB.

A third conclusion is that in at least one case, the C protein from one GerA receptor homolog can interact with the A and B proteins of another receptor. While this was not the case for GerAC and GerBA-GerBB, high levels of $GerBC^{Cys20}$ significantly decreased GerA receptor function. The simplest explanation for this observation is that high levels of $GerBC^{Cys20}$ compete well with $GerAC^{Cys18}$ for binding to GerAA-GerAB, and the presence of $GerBC^{Cys20}$ in this complex generates an inactive receptor. This explanation is consistent with the inter-

action of GerAC with GerAA-GerAB being not extremely strong, as suggested above. Presumably, GerBC interaction with GerBA-GerBB is much stronger, which is why GerAC overexpression does not alter GerB receptor function. However, the interaction of GerBC with GerAA-GerAB may not be physiologically significant, as it was seen only with high levels of GerBC^{Cys20}. The possibility of interactions among different GerA receptor homologs is also worth noting. Recent work on the plasma membrane chemoreceptors that modulate prokaryotic chemotaxis has shown that formation of large multireceptor complexes is important in the function of these receptors (11, 27, 28). Perhaps spore nutrient receptors are another example of this phenomenon.

The fourth conclusion is that *gerAC*^{Ala18}, *gerBC*^{Cys20}, and perhaps *gerBC*^{Ala20} can act as dominant negative mutants, as was seen in the marked reduction in the GerA-mediated germination of spores of the strain that overexpresses *gerAC*^{Ala18} at *amyE* and in the significant decrease in GerB*-mediated germination of spores with high levels of GerBC^{Cys20}. The simplest interpretation of these results and those supporting the other conclusions is that GerAC physically interacts with GerAA-GerAB, while GerBC physically interacts with GerBA-GerBB. As noted above, there is evidence for direct interaction between GerAA and GerAB and between GerBA and GerBB (15, 18). It is possible that the three GerA and GerB proteins do not form a long-lived complex, but associate only transiently as they diffuse through the dormant spore's inner membrane. However, since lipid probes appear to be largely immobile in the dormant spore's inner membrane (3), it is difficult to imagine that proteins readily diffuse in this membrane, although this could be the case in developing forespores. Consequently, it appears likely that GerAA-GerAB-GerAC and GerBA-GerBB-GerBC form a long-lived complex in the spore's inner membrane. Given the unusual properties of this membrane (3), further understanding of the function of the nutrient receptors will require knowledge of their structure and knowledge of the proteins with which the receptors interact.

This work was supported by grant GM19698 from the National Institutes of Health.

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